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EXAMINER
RAWLINGS, STEPHEN L

ART UNIT	PAPER NUMBER
1642	

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Please find below and/or attached an Office communication concerning this application or proceeding.

Office Action Summary	Application No.	Applicant(s)	
	09/555,211	STEINLEIN ET AL.	
	Examiner	Art Unit	
	Stephen L. Rawlings, Ph.D.	1642	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Peri d for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE 3 MONTH(S) FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If the period for reply specified above is less than thirty (30) days, a reply within the statutory minimum of thirty (30) days will be considered timely.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133).
- Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *21 November 2001* .

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *19-45* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *19-45* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

11)☐ The proposed drawing correction filed on _____ is: a)☐ approved b)☐ disapproved by the Examiner.

If approved, corrected drawings are required in reply to this Office action.

12)☐ The oath or declaration is objected to by the Examiner.

Pri rity under 35 U.S.C. §§ 119 and 120

13)☒ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☒ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____ .

3.☒ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

14)☐ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. § 119(e) (to a provisional application).

a) ☐ The translation of the foreign language provisional application has been received.

15)☒ Acknowledgment is made of a claim for domestic priority under 35 U.S.C. §§ 120 and/or 121.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO-1449) Paper No(s) _____ .
4) ☐ Interview Summary (PTO-413) Paper No(s). _____ .
5) ☐ Notice of Informal Patent Application (PTO-152)
6) ☐ Other: .

DETAILED ACTION

1. The amendment and declaration under 37 CFR § 1.132 filed November 21, 2001 in Paper No. 10 is acknowledged and has been entered. Claims 29-31 and 35-37 have been amended.

2. Receipt of the English translation of the certified copy of the foreign priority document, namely the German Patent Application No. 197 52 922.4, on November 21, 2001 is acknowledged and has been entered as Paper No. 11.

3. Claims 19-45 are pending in the application and are currently under prosecution.

Response to Amendment and Applicants' Remarks

4. In response to the previous Office Action Applicants have submitted the English translation of the foreign priority document, namely the German Patent Application No. 197 52 922.4, which was filed on November 28, 1997, thereby obviating the grounds of the rejection of the claims under 35 USC § 103 for the reason stated in the previous Office Action. Therefore, the rejection of claims 19-45 under 35 USC § 103(a) for the reason stated in the previous Office Action is withdrawn.

The Declaration under 37 CFR § 1.132 by Peter Steinlein filed in Paper No. 10 satisfactorily clarifies the inventorship of the claimed invention; therefore, the rejection of claims 19-45 under 35 USC § 102(f) for the reason stated in the previous Office Action is withdrawn.

Applicants state that in view that all stated grounds of objection and rejection have been properly traversed, accommodated, or rendered moot, the present application is in condition for allowance. Applicant's arguments with

respect to claims 19-45 have been considered but are moot in view of the new ground(s) of rejection.

Specification

5. The use of the numerous trademarks has been noted in this application. Each letter of a trademark should be capitalized or otherwise the trademark should be demarcated with the appropriate symbol indicating its proprietary nature (e.g., ™, ©, ®), and accompanied by generic terminology.

Although the use of trademarks is permissible in patent applications, the proprietary nature of the marks should be respected and every effort made to prevent their use in any manner that might adversely affect their validity as trademarks. See MPEP § 608.01(v).

Claim Objections

6. Claims 19-45 are objected to because of the following informalities:

(a) Claims 19-45 are objected to because "fluorescent" is consistently misspelled throughout the claims. Appropriate correction is required.

(b) Claims 30 and 36 are objected because "fibroblast" is misspelled. Appropriate correction is required.

(b) Claim 39 is objected to as being improper. A claim that depends from a dependent claim should not be separated by any claim that does not also depend from said dependent claim. See MPEP § 608.01(n). Appropriate correction is required.

(c) Claim 45 is objected to because the claim recites the phrase "FACS sorting" in line 15. Recitation of "FACS" with "sorting" is redundant since

the acronym is used to identify "fluorescence-activated cell sorting". Appropriate correction is required.

Claim Rejections - 35 USC § 112

7. The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

8. Claims 19-25 and 45 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 19-25 are drawn to a method for determining the proportion of apoptotic cells in a culture. However, if one were to perform the claimed method according to the steps included in the body of the claim, one could not determine the proportion of apoptotic cells that were originally present in the culture, because the method measures the proportion of apoptotic cells that have been co-transfected with a polynucleotide sequence encoding and presumably capable of expressing, a fluorescent protein and another undisclosed polynucleotide sequence, which can encode and presumably would be capable of expressing, a protein that is suspected of having an activity that influences the onset of apoptosis in cells. The cells altered by transfection are not representative of the population of cells obtained from the original culture. The specification does not teach a method for determining whether cells in a culture are undergoing apoptosis. For this reason, with regard to claims 19-25, the subject matter of the claims is not described in the specification in such a way as to enable one skilled in the art to practice the claimed invention.

However, it appears that the specification teaches a method for determining whether or not a candidate polynucleotide sequence encodes a

protein that is affects the onset or rate of onset of apoptosis in a cell transfected with the sequence when the cell is cultured under conditions that allow the protein to be expressed in the cell. Co-transfection of the cell with another polynucleotide sequence that encodes and expresses a fluorescent protein serves to mark the transfected cells, which are those cells in the original population of cells that have incorporated the polynucleotide sequences. If the ratio of the quantities of the polynucleotide sequence encoding the fluorescent protein and the candidate polynucleotide sequence is sufficiently high, the majority of cells, which express the fluorescent protein, will have incorporated both polynucleotide sequences and therefore the majority of fluorescent cells will also express the candidate protein. The transfected cells marked by their fluorescence can then be separately quantified by flow cytometric analysis of the transfected cell culture. Methods for quantifying the number of cells undergoing apoptosis in a given population of cells by flow cytometric means are routine and conventional. Flow cytometry enables analysis of a electronically selected or *gated* population of cells, which can be enumerated and sorted upon the basis of the cells' fluorescent properties, so that the percentage of cells undergoing apoptosis in a population of fluorescent cells, i.e., in this case, the transfected cells, can be measured. Since the cDNA encoding Green Fluorescent Protein (GFP) was cloned from the jellyfish *Aequorea victoria* in 1994, it has been widely used as a reporter gene to mark transfected cells. Since 1994, several variants of GFP have been engineered, which have enhanced properties that facilitate improved analysis and sorting of transfected cells expressing the fluorescent proteins by flow cytometry and fluorescent microscopy.

While the methods described in the paragraph above are largely conventional, claim 45 is drawn to a method for expression cloning a gene that modulates apoptosis, but it is unclear that the method can be used to clone any gene that modulates apoptosis and the specification does not exemplify the successful use of the claimed invention. The method comprises many of the same steps discussed in the paragraph above. Indeed, it would be possible to

isolate a single cell or cells that have distinctive fluorescent properties, such as cells that produce a fluorescent protein. However, as cells undergo the process of apoptosis or during development, the process called programmed cell death, DNA-nicking enzymes that digest the genetic material (i.e., DNA) in a cell are produced, which rapidly cleave the DNA into small pieces containing only fragments of the genes that were contained with the intact DNA molecules. DNA degradation is one of the most recognizable hallmarks of a cell that is undergoing the apoptotic process. As a cell progresses toward its own final destruction, the membrane of the cell becomes permeable so that larger molecules, which would normally be retained by the membrane, can traverse the membrane to leak from the interior of the cell. In this way, during apoptosis, the small fragments of the cell's DNA molecules diffuse out of the cell. It is the decreased quantity of DNA in the interior of the apoptotic cell, which when measured by conventional techniques, enables one to distinguish the apoptotic cells from other cells in the same population that were not undergoing apoptosis. If a cDNA molecule encoding a protein that induces apoptosis in a cell, once the protein is expressed, is transiently transfected into a cell, the cell will express the protein and the protein will induce the onset of apoptosis in the cell. As the process apoptosis ensues, the DNA in the cell, including the cDNA molecule encoding the protein will be degraded. It is not apparent that a cDNA molecule encoding a protein that induces the cell's apoptosis, once the cDNA molecule is transfected into the cell, can be isolated from transfected cells before the cDNA is degraded. Therefore, as the claims are now written, it is unclear that the invention can be practiced with a reasonable expectation of success, particularly in the absence of working exemplification of the claimed method. Because the method is not exemplified, one skilled in the art would have to perform undue experimentation to determine if the invention can be used with a reasonable expectation of success.

Additionally, it is noted that the apoptotic process is an extremely complex biologic phenomenon, which has yet to be fully described and understood. The

is an enormously diverse group of genes and proteins that have been found to affect apoptosis in cells, many of these gene's expression are regulated by exquisitely complex mechanisms and many of these proteins have markedly different functions. Therefore, the specification provides insufficient guidance to enable the skilled artisan to conduct expression and function studies to characterize the protein encoded by a cDNA molecule. For these reasons, the subject matter of claim 45 is not described in the specification in such a way as to enable one skilled in the art to practice the claimed invention.

9. The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

10. Claims 19-45 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 19-25 and 45 are indefinite because claims 19 and 45 do not recite a positive process step that clearly relates back to the preamble of the claim. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. Amending claim 19 to recite, for example, the phrase, "whereby the proportion of apoptotic cells in a culture is determined" at the end of the last line of the claim can obviate this rejection insofar the rejection pertains to claim 19. Similarly, amending claim 45 to recite, for example, the phrase "whereby a gene that modulates apoptosis is cloned" at the end of the last line of the claim can obviate this rejection insofar the rejection pertains to claim 45.

Claims 19-25 are indefinite because claim 19 recites the phrase "[a] method of determining the proportion of apoptotic cells in a culture". Recitation of the phrase renders the claim indefinite because it cannot be ascertained to which culture the claim refers. Nevertheless, at first glance, it appears that none of the steps included in the body of claim determine the proportion of apoptotic

cells in the cells of a culture, because the steps involve altering some or all of the cells in the culture and then only determining the proportion of the altered cells in the culture that are undergoing apoptosis, but not determining the proportion of the unaltered cells, which would be representative of the original culture to which the preamble of the claim refers. Because the claim is indefinite one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. However, as noted in the 35 USC § 112, first paragraph rejection above, the steps collectively recited in the claim appear to form a process for determining whether a sequence of interest has an effect upon the incidence of apoptosis in a population of cells transfected with the sequence of interest. Therefore, amending the preamble of the claim to recite, for example, "A method for determining transiently transfecting cells with a sequence of interest affects apoptosis of the transfected cells, said method comprising ..."; but Applicant is cautioned against the introduction of new matter.

Claims 19-40 and 45 are indefinite because claims 19, 26, 32, and 45 recite the term "suitable nutrient medium". Recitation of the term renders the claims indefinite because "suitable" is a relative term, which is not defined by the claim. Because the specification does not provide a standard for ascertaining the requisite degree of suitability, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. Amending claims 19, 26, 32, and 45 to delete "suitable" can obviate this rejection.

Claims 19-25 are indefinite because claim 19 recites the limitation "the DNA sequence of interest" in line 6. There appears to be insufficient antecedent basis for recitation of this limitation in the claim. Amending the claim to delete "DNA" can obviate this rejection.

Claims 19-40 and 45 are indefinite because claims 19, 26, 32, and 45 recite the limitation "the apoptotic DNA fragments". There appears to be insufficient antecedent basis for recitation of this limitation in the claim. Amending the claim to delete "the" in the limitation can obviate this rejection.

Claims 19-31 and 45 are indefinite because claims 19, 26, and 45 recite the phrase "so that the DNA sequence of interest or its expressed polypeptide exerts its potential activity". Recitation of the phrase renders the claim indefinite because it is unclear if a potential activity can be exerted. If one defines "potential" as "existing in possibility or capable of development into actuality", then once an activity is exerted by the sequence of interest or the expressed polypeptide, the activity should no longer be described as potential. Because the specification does not provide a standard for ascertaining the requisite degree of suitability, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. Amending claims 19, 26, and 45 to delete "potential" can obviate this rejection.

Claims 19-40 and 45 are indefinite because claims 19, 26, 32, and 45 recite the phrase "so that **any** [fluorescent] protein expressed remains in the cells" (emphasis added). It is unclear that every molecule of the fluorescent protein (i.e., any one) present in the cell can be retained; some of the molecules may not be retained in the cells. Also, during the process of apoptosis, a cell forms apoptotic bodies, which are fragments of cells; therefore, the method is more likely to only retain fluorescent protein in the harvested cells that remain intact. Because of the uncertainty, the claim is indefinite and one of ordinary skill in the art would therefore not be reasonably apprised of the metes and bounds of the invention. Amending claims 19, 26, 32, and 45 to recite, for example, the phrase "so that most fluorescent protein expressed in the cells remaining intact is retained" could obviate this rejection.

Claims 19-40 and 45 are vague and indefinite because claims 19, 26, 32, and 45 recite the phrase "measuring the proportion of apoptotic cells by measuring total DNA content remaining subsequent to [step] (D)". Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim would require the practitioner to measure the proportion of apoptotic cells by measuring total DNA content of the cells. Accordingly, one of ordinary skill in the art would not be reasonably apprised of

the metes and bounds of the claims. It would appear that the disclosure teaches that the proportion of apoptotic cells can be determined by measuring the proportion of hypodiploid (i.e., having a DNA content of < 2 N) cells. Therefore, it is suggested that amending claims 19, 26, 32, and 45 to recite, for example, the phrase "measuring the proportion of the harvested cells containing a DNA content of < 2 N and thereby determining the proportion of the harvested cells that were apoptotic at the time the measurement was made" can obviate this rejection; but again, Applicant is cautioned against the introduction of new matter.

Claims 19-40 and 45 are vague and indefinite because claims 19, 26, 32, and 45 recite the phrase "measuring the proportion of transfected cells by quantitating the [fluorescent] marker protein contained in the cells harvested" in lines 14 and 15. Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how determining the quantity of the fluorescent marker protein contained in the harvested cells can be used to measure the proportion of transfected cells. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims. However, measuring the percentage of cells containing detectable quantities of fluorescent protein would be expected to provide a determination of the percentage of the cells that were transfected. Therefore, it is suggested that amending claims 19, 26, 32, and 45 to recite, for example, the phrase "measuring the proportion of the harvested cells containing fluorescent marker protein and thereby determining the proportion of the harvested cells transfected with the sequence of interest" can obviate this rejection; but again, Applicant is cautioned against the introduction of new matter.

Claims 19-40 are vague and indefinite because claims 19, 26, and 32 recite the phrase "comparing the values" and/or the phrase "comparing the calculated proportion of apoptotic cells". Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim requires the values to be compared. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claims 19-25 are vague and indefinite because claim 19 recites the phrase "determining the proportion of apoptotic cells in the transfected population" in line 16. Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim requires the proportion of apoptotic cells in the transfected population is to be determined. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claim 24 is vague and indefinite because the claim recites the limitation "wherein the fixing and permeabilization in (D) is achieved with paraformaldehyde and ethanol, respectively" without reciting the specific conditions that are used to achieve the result. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims. Amending the claim to recite the specific condition under which the cells are treated with paraformaldehyde and ethanol can obviate this rejection. Alternatively, amending the claim to recite, for example, the phrase "wherein the fixing and permeabilization in (D) is achieved **using** paraformaldehyde and ethanol, respectively" (emphasis added for illustration) can obviate the rejection.

Claims 26-31 are vague and indefinite because claim 26 recites the phrase "determining whether the gene of interest affects the proportion of apoptotic cells in the transfected population" in lines 23 and 24. Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim requires the practitioner to determine whether the gene of interest affects the proportion of apoptotic cells in the transfected population. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claims 27-31 and 33-37 are indefinite because claims 27, 28, 33, and 34 recite the term "particular to a tumor cell". Recitation of the term renders the claims indefinite because the term is not defined in the claim or appears not to be defined in the specification. It cannot be ascertained whether the claim requires the survival factor to be a distinguishing feature of only the single tumor cell to

which the claim refers or in all tumor cells or in only some tumor cells, or not in non-tumor cells. In other words, it is unclear how the claim requires the survival factor to be particular to a tumor cell. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention.

Claims 27, 33, and 39 are vague and indefinite because claims 27 and 33 recite the term "signal transmission molecule of a receptor". Recitation of the term renders the claims vague and indefinite because the term is not defined by the claim, nor is the term explicitly in the specification. While the specification discloses example of molecules that are considered to be "signal transmission molecule of a receptor", because the term is not defined, it is still not apparent which molecules are encompassed by the claims, and which are not. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention.

Claims 32-40 are indefinite because claim 32 recites the limitation "those test substances" in line 22. There appears to be insufficient antecedent basis for recitation of this limitation in the claim.

Claims 32-40 are indefinite because claim 32 recites the step "selecting those test substances which exhibit a synergistic activity". Recitation of the phrase step renders the claim indefinite because it cannot be determined to which activity of the test substances the claim refers. It cannot be determined whether the test substances are required to "synergize" with another test substance or alternatively, with some other undisclosed entity. Also, the claim does not recite a step in which a second, third, fourth, etc. test substance is used and so it is not clear to which other test substances the claim refers. Furthermore, since a test substance can have either pro- or anti-apoptotic activity, it is unclear what is meant by the term "exhibits *synergistic* activity" (italics added for emphasis). Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention.

Claims 32-40 are vague and indefinite because claim 32 recites the phrase "determining whether the gene of interest affects the proportion of

apoptotic cells in the transfected population" in lines 23 and 24. Recitation of the phrase renders the claim vague and indefinite because it cannot be ascertained how the claim requires the practitioner to determine whether the gene of interest affects the proportion of apoptotic cells in the transfected population. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claims 41-44 are indefinite because claim 41 and 42 recite the term "sufficient". Recitation of the term renders the claims indefinite because it cannot be ascertained whether the claim requires each and every one of the reagents needed to perform the transfection to be contained in the kit or alternatively, a sufficient amount of one or more of the reagents to be contained in the kit. The specification does not provide a standard for ascertaining the requisite quantity of each reagent that is to be considered sufficient. Therefore, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the invention. Amending claim 41 to delete "suitable" can obviate this rejection insofar as the rejection pertains to claim 41.

Claims 41-44 are indefinite because claim 41 recites the limitations "wherein: (A) the first container" in line 4, "the [fluorescent] marker protein" in lines 5 and 6, "the primary fixing solution" in line 9, and "the secondary fixing/permeabilizing solution" in line 10. There appears to be insufficient antecedent basis for recitation of these limitations in the claim.

Claims 41-44 are indefinite because claim 41 recites the term "container means" in line 2 and then the term "container" in lines 4, 5, 7, and 9-12. It is unclear whether the "container" is the same as a "container means" to which the claim refers in the preamble. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claim 42 is indefinite because the claim recites the limitation "wherein the transfection components". There appears to be insufficient antecedent basis for recitation of this limitation in the claim from which claim 42 depends, i.e., claim

41. Amending claim 42 to recite, for example, the limitation "wherein the components for transfection are [...]" can obviate this rejection.

Claim 44 is indefinite because the claim recites the terms "2% paraformaldehyde" and "70% ethanol". Recitation of the terms renders the claims indefinite because it cannot be determined if the concentrations are expressed at unit mass/unit volume or unit volume/unit volume. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claim 45 is indefinite because the claim recites the limitation "the population of cells" in line 3. There appears to be insufficient antecedent basis for recitation of the limitation in the claim.

Claim 45 is vague and indefinite because the claim recites the phrase "single cells which deviate from an apoptosis background which is to be determined". Recitation of the phrase renders the claim vague and indefinite because: (a) the term "apoptosis background" is not defined by the claim and it cannot be determined to what measurement the claim refers, (b) it cannot be determined when or how the claim requires the "apoptosis background" to be measured, and (c) it cannot be determined how and to what extent the claim requires the single cells to deviate from an "apoptosis background", so it cannot be determined which cells the claim requires to be isolated. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claim 45 is vague and indefinite because the claim recites the step "isolating, amplifying, and selecting the transfected plasmids in a further transfection process". Recitation of the phrase renders the claim indefinite because: (a) it cannot be determined to what further transfection process the claim refers and (b) it cannot be determined how the claim requires the transfected plasmids to be selected after isolating and amplifying the plasmids. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claim 45 is vague and indefinite because the claim recites the step "characterizing the corresponding genes on the plasmids isolated and amplified in [step] (H) by sequencing and conducting expression and function studies". Recitation of the phrase renders the claim indefinite because: (a) it cannot be determined how the claim requires the corresponding genes on the plasmids to be characterized by sequencing and conducting expression and function studies, (b) it cannot be determined how or to what entity or entities the claim requires the genes on the plasmids to correspond, and (c) it cannot be determined which expression and function studies the claim would require the practitioner to conduct. Accordingly, one of ordinary skill in the art would not be reasonably apprised of the metes and bounds of the claims.

Claim Rejections - 35 USC § 102

11. The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless –
>
> (a) the invention was known or used by others in this country, or patented or described in a printed publication in this or a foreign country, before the invention thereof by the applicant for a patent.
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.
>
> (e) the invention was described in-
>
> > (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effect under this subsection of a national application published under section 122(b) only if the international application designating the United States was published under Article 21(2)(a) of such treaty in the English language; or
> >
> > (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that a patent shall not be deemed filed in the United States for the purposes of this subsection based on the filing of an international application filed under the treaty defined in section 351(a).

12. Claims 19, 21, 22, 23, 25, and 26 are rejected under 35 U.S.C. 102(a) as anticipated by or, in the alternative, under 35 U.S.C. 103(a) as obvious over Keane, et al (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* **38**: A1148, 1997, Abstract No. 1148).

With view of the discussion set forth in the 35 USC § 112, first paragraph rejection, claims 19, 21, 22, 23, 25, and 26 are examined insofar as the claims are drawn to a method for measuring the proportion of apoptotic cells in a culture of cells co-transfected with a sequence of interest and a plasmid containing a polynucleotide sequence encoding a fluorescent protein to determine whether the sequence of interest encodes a protein that affects apoptosis in the transfected cells. Keane, et al teach a method for determining whether DEP-1, i.e., the sequence of interest, encodes a protein that induces apoptosis in breast cancer cells by process that involves measuring the proportion of cells transfected with a cDNA molecule encoding DEP-1 protein that undergo apoptosis and comparing the value of that proportion to the value of the proportion of cells undergoing apoptosis that were transfected with a molecule that does not encode DEP-1 protein. According to the method of Keane, et al the breast cancer cells are transfected with a vector encoding DEP-1 protein. The cells are harvested and apoptosis is assessed using propidium iodide staining. Cells are analyzed by flow cytometry. A process that involves measuring the total DNA content of the harvested cells thus determines the proportion of apoptotic cells. Permitting slight but insignificant variations, the steps, which are not explicitly taught by the reference, are understood to be necessary to perform the analysis by the method taught by the reference and are accordingly considered intrinsic to the method. While Keane, et al do not explicitly state that the harvested cells were fixed and permeabilized, so that apoptotic DNA fragments diffuse out of the cells before the proportion of apoptotic cells is measured, because the method used by Keane, et al to measure apoptosis was conventional in the art and the scientific basis for the steps of fixing and permeablilizing the cells was well understood, it would have been *prima facie*

obvious to one of ordinary skill in the art at the time the invention was made to have fixed and permeabilized the harvested cells before measuring the proportion of apoptotic cells. Keane, et al teach, "[t]ransfected cells were then selected by co-transfection with green fluorescent protein" to assess the effect of DEP-1 protein expression in transfected cells only. While it is not explicitly stated in the reference that propidium iodide staining was used to assess the level of apoptosis in the selected population of transfected cells, since Keane, et al may have used the TUNEL assay instead, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use propidium iodide staining to measure the proportion of apoptotic cells in the selected population of transfected cells by gating on the fluorescent cells during analysis of the data generated by flow cytometry. Therefore, if the limitations of the claims are not explicitly anticipated by the teachings of Keane, et al, in further view of the state of the art at the time the invention was made, it would have been *prima facie* obvious to one of ordinary skill in the art at the time to derive the instant invention. One of ordinary skill in the art at the time would have been motivated to derive the instant invention, because Keane, et al teach that two distinct methods for measuring apoptosis can yield slightly different results and thereby illustrate the utility of confirming the results of one assay with another, in order to be scientifically thorough and make sound conclusions regarding the implication of the data.

13. Claims 19, 21, 22, 23, and 25-28 are rejected under 35 U.S.C. 102(a) as being anticipated by Kalejta, et al (*Cytometry* **29**: 286-291, 1997).

With view of the discussion set forth in the 35 USC § 112, first paragraph rejection, claims 19, 21, 22, 23, and 25-28 are examined insofar as the claims are drawn to a method for measuring the proportion of apoptotic cells in a culture of cells co-transfected with a sequence of interest and a plasmid containing a polynucleotide sequence encoding a fluorescent protein to determine whether

the sequence of interest encodes a protein that affects apoptosis in the transfected cells.

Kalejta, et al teach a method for determining the proportion of cells transfected with a sequence of interest that undergo apoptosis as a result of expressing the protein encoded by the sequence of interest. According to the method of Kalejta, et al cells are co-transfected with an expression plasmid encoding Green Fluorescent Protein (GFP) and another expression plasmid comprising a sequence of interest (page 287, columns 1 and 2); the latter of which encodes either wild-type p53 protein or a dominant negative mutant p53 protein that does not bind DNA (page 289, column 2). Alternatively, cells are co-transfected with an expression plasmid encoding Green Fluorescent Protein (GFP) and a control plasmid. The cells are harvested, fixed, and/or permeabilized, the DNA in the cells is stained with propidium iodide, and the apoptotic proportion of cells is determined by analysis of data acquired by the simultaneous measurement of GFP and propidium iodide fluorescence using a flow cytometer (page 287, column 2). Kalejta, et al teach, "[the method] may be employed to quantitate apoptosis by the appearance of a sub-G0/G1 peak by flow cytometry and to investigate the role of specific proteins on the apoptotic process" (page 290, column 2).

Although Kalejta, et al does not explicitly disclose that the mutant p53 protein encoded by the co-tranfected expression plasmid is a "dominant negative signal transmission molecule of a receptor for a survival factor particular to a tumor cell" or a "dominant negative receptor for a survival factor particular to a tumor cell", it is well known in the art that p53 protein is intricately involved in determining the survival of a cell by regulating apoptosis and has been shown to function as both a receptor for survival factors particular to tumor cells and a signal transmission molecule of a receptor for a survival factor particular to a cell. For a recent review of the signal transduction pathways that regulate the activity of p53 and the pathways that are induced by p53, Applicants are referred to Balint, et al (*British Journal of Cancer* **85**: 1813-1823, 2001). Since the sequence

of interest of the prior art is deemed the same as the sequence of interest of the claims that encodes a dominant negative signal transmission molecule of a receptor for a survival factor particular to a tumor cell or a dominant negative receptor for a survival factor particular to a tumor cell, the method of the prior art is deemed the same as the method of the claims.

All the limitations of the claims are thus met.

14. Claims 19, 21, 22, 23, 25, and 26 are rejected under 35 U.S.C. 102(e) as being anticipated by US Patent No. 5,976,853 A.

With view of the discussion set forth in the 35 USC § 112, first paragraph rejection, claims 19, 21, 22, 23, and 25-28 are examined insofar as the claims are drawn to a method for measuring the proportion of apoptotic cells in a culture of cells co-transfected with a sequence of interest and a plasmid containing a polynucleotide sequence encoding a fluorescent protein to determine whether the sequence of interest encodes a protein that affects apoptosis in the transfected cells.

US Patent No. 5,976,853 A teaches a method for measuring the proportion of apoptotic cells in a culture of cells co-transfected with a sequence of interest and a plasmid containing a polynucleotide sequence encoding a fluorescent protein to determine whether the sequence of interest encodes a protein that affects apoptosis in the transfected cells. According to the method of '853 cells are co-transfected with an expression plasmid encoding Green Fluorescent Protein (GFP) and another expression plasmid comprising a sequence of interest, which encodes FIN13 protein, or alternatively a control plasmid. Following transfection, cells were harvested and sorted for GFP-positive cells by fluorescence-activated cell sorting (FACS) using a flow cytometer. Recovered cells were fixed, stained with propidium iodide, and analyzed for DNA content by flow cytometry (see, for example, Figure 7 and column 8, lines 43-53). Based upon the results of a study utilizing the method,

'853 teaches, "the primary effect of FIN13 was not to induce programmed cell death [i.e., apoptosis]" (columns 52 and 53).

All the limitations of the claims are thus met.

Claim Rejections - 35 USC § 103

15. The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

16. Claims 19-44 are rejected under 35 U.S.C. 103(a) as being unpatentable over Keane, et al (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* **38**: A1148, 1997, Abstract No. 1148) in view of Douglas, et al (*Journal of Immunological Methods* **188**: 219-228, 1995), Anderson, et al (*Proceedings of the National Academy of Science USA* **93**: 8508-8511, 1996), and Baker, et al (*Nucleic Acids Research* **25**: 1950-1956) and in further view of Keane, et al (*Proc. Annu. Meet. Am. Assoc. Cancer Res.* **37**: A299, 1996; Database CANCERLIT, Accession No. 96647249) or in still further view of Sell, et al (*Cancer Research* **55**: 303-306, 1995) and Prager, et al (*Journal of Clinical Investigation* **90**: 2117-2122, 1992) or Chow, et al (*Development* **121**: 4383-4393, 1995) or Strawn, et al (*Journal of Biological Chemistry* **269**: 21215-21222, 1994) or Trent, et al (*EMBO Journal* **15**: 4497-4505, 1996), as evidenced by the teachings of Chu, et al (*Cytometry* **36**: 333-339, 1999).

Keane, et al (1997) teach that which is set forth in the corresponding 35 USC §§102/103 rejection above. However, Keane, et al do not explicitly teach that the harvested cells can be fixed and permeabilized with paraformaldehyde and ethanol, respectively, before measuring the proportion of apoptotic cells by measuring the total DNA content of the cells by flow cytometry. Furthermore,

Keane, et al do not teach that the transient transfection of the cells can involve receptor-mediated endocytosis by a method that involves using polyethyleneimine and inactivated adenovirus.

However, Douglas, et al teach a method for determining the proportion of apoptotic cells that express a particular marker protein at the cell's surface. According to the method of Douglas, et al, in preparation of the cells for staining with a DNA binding stain, the cells are harvested and fixed with paraformaldehyde and permeabilized. Alternatively, the cells can be fixed and permeabilized with ethanol. After staining the DNA in the cells, analytical cytometry using a flow cytometer is performed to determine the proportion of cells undergoing apoptosis. Douglas, et al teach, "apoptosis in freshly isolated murine lymphocytes detected with paraformaldehyde fixation and PI [propidium iodide] staining was quantitatively comparable to PI staining with ethanol fixation, or nick translation labeling of DNA strand breaks (TUNEL)" (abstract). While Douglas, et al do not teach that cells expressing Green Fluorescent Protein can be determined during analysis of apoptosis, because Douglas, et al teach that the method can be used to coordinately examine apoptosis and the presence of FITC-stained cell surface markers and because a cDNA molecule encoding Green Fluorescent Protein (GFP) had been widely used as a reporter gene in transfection studies, as evidenced by the teachings of Anderson, et al, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have adapted the method of Douglas, et al to coordinately examine the proportion of apoptosis in transfected cells marked by the presence of GFP. One of ordinary skill in the art would have been motivated to use GFP as a marker rather than a cell surface protein or β-galactosidase, because in doing so, one would gain the advantage of not having to use an antibody against the cell surface marker or treat the cells with substrate to stain the transfected cells. Because GFP auto-fluoresces, no other costly reagents would be required to practice the method.

Baker, et al teach a method for transfecting plasmids into cells using polyethyleneimine (PEI) and psoralen-inactivated adenovirus. Baker, et al teach that the use of the method offers a number of advantages over the use of other methods (see, for example, page 1953, Figure 2; page 1956, column 1).

It would therefore have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have combined the method of Keane, et al (1997) and Baker, et al because Baker, et al teach that higher levels of transfection can be achieved using their adenovirus transfection system than with other conventional transfection systems and one would have been motivated to achieve as high a transfection efficiency as possible, because it is desirable to do so. Furthermore, on the basis of the teachings of Douglas, et al, it would have been prima facie obvious to one of ordinary skill in the art to have fixed and permeablilized the cells before analysis using paraformaldehyde and ethanol, respectively, because Douglas, et al teach that paraformaldehyde can be used to fix the cells before permeabilization and it was already known that ethanol could be used to permeabilize the cells. One would have been motivated to fix the cells before analysis so as to prevent GFP from leaching out of the cells after permeabilization and one would have been motivated to permeabilize the cells because it was already established that permeabilization of the cells enables small apoptotic DNA fragments to diffuse out of the cells, thus creating the signature hypodiploid state, which distinguishes the apoptotic cell from the non-apoptotic cell. Moreover, one would have had a reasonable expectation of success in practicing the method for determining the proportion of apoptotic cells transfected with an expression vector encoding GFP, as evidenced by the teachings of Chu, et al.

However, neither Keane, et al, Douglas, et al, Anderson, et al, or Baker, et al explicitly teach that the sequence of interest can encode a dominant negative signal transmission molecule of a receptor for a survival factor particular to a tumor cell or alternatively a dominant negative receptor for a survival factor particular to a tumor cell.

Keane, et al (1996) teach a mutant of a sequence of interest, namely a catalytically inactive or dominant negative version of DEP-1 protein, which Keane, et al teach does not inhibit clone forming ability in three breast cancer cell lines. By contrast, Keane, et al teach that wild-type DEP-1, which is catalytically active, inhibits the growth of the breast cancer cell lines *in vitro*, but Keane, et al did not disclose that the protein inhibits apoptosis by inducing apoptosis.

In view of the teachings of Keane, et al (1996), it would have been *prima facie* obvious to one of ordinary skill in the art to use a polynucleotide sequence encoding the dominant negative version of DEP-1 protein as the sequence of interest in practicing the method for determining the proportion of apoptotic cells transfected with the sequence of interest. One of ordinary skill in the art would have been motivated to use the polynucleotide encoding the dominant negative version of DEP-1 protein as the sequence of interest, because it would have been important to determine the mechanism by which expression of DEP-1 inhibits the growth of tumor cells and therefore it would have been desirable to compare the effects of transfecting a tumor cell with the plasmid encoding wild-type DEP-1 and transfecting a tumor cell with the plasmid encoding the dominant negative version of DEP-1 protein.

However, neither Keane, et al (1996 and 1997), Douglas, et al, Anderson, et al, or Baker, et al explicitly teach that the sequence of interest can encode a dominant negative insulin-like growth factor I (IGF-1) receptor, a dominant negative fibroblast growth factor (FGF) receptor, or a dominant negative platelet-derived growth factor (PDGF) receptor. Also, none of these cited references teach that the sequence of interest can encode a dominant negative signal transmission molecule of a receptor for a survival factor particular to a tumor cell, such as dominant negative Ras.

Sell, et al teach that IGF-I receptor and IGF-1 prevent etoposide-induced apoptosis. Prager, et al teach that human IGF-1 receptor function in cells can be suppressed by expression of a dominant negative version of the IGF-I receptor in the cells.

Chow, et al teach a dominant negative version of the FGF receptor, which was developed to study the role of FGF in the development of the lens. Chow, et al teach that FGF suppresses apoptosis in the lens.

Strawn, et al teach that the growth of glioma cells can be inhibited by expressing a dominant negative version of PDGF receptor in the cells.

Trent, et al teach that a signaling pathway involving activation of Ras protein is required for tumor necrosis factor (TNF)-induced apoptosis. Trent, et al also teach a dominant negative version of Ras protein, which Trent, et al teach can be used to study the mechanism by which expression of Ras in a cell sensitizes the cell to apoptosis upon exposure to TNF.

In view of the teachings of Sell, et al and Prager, et al, Chow, et al, Strawn, et al, or Trent, et al, it would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to have substituted a polynucleotide encoding the IGF-I receptor or a dominant negative version thereof, a polynucleotide encoding the FGF receptor or a dominant negative version thereof, a polynucleotide encoding the PDGF receptor or a dominant negative version thereof, or a polynucleotide encoding the Ras or a dominant negative version thereof, respectively, for the sequence of interest of Keane, et al, namely the polynucleotide encoding DEP-1 protein or the dominant negative version thereof. One of ordinary skill in the art would have been motivated make one or the other substitution in order to study the role of the IGF-I, FGF, or PDGF receptor or the role of Ras in apoptosis of tumor cells, because while each had been determined to play such a role, the precise mechanisms by which the proteins did so had not been completely elucidated. Moreover, because the expression of the proteins had been associated with a tumor's resistance to chemotherapy, e.g., TNF or etoposide therapy, it would have been *prima facie* obvious to one of ordinary skill in the art to use the method to determine the effect of candidate chemotherapeutic agents in tumor cells expressing either the wild-type protein or a dominant negative version of the protein, because one would have been motivated to characterize the effect of the agents and assess

whether expression of the protein synergizes or antagonizes the effect of the agent.

Finally, none of cited references explicitly teach a kit comprising some of the reagents, namely one or more components for use in transfecting cells, particularly sufficient component to achieve receptor-mediated endocytosis using polyethleneimine and psoralen/UV-inactivated adenovirus; a plasmid containing a sequence encoding a fluorescent protein, particularly one encoding Green Fluorescent Protein; an empty vector for use in cloning a sequence of interest and as a control; a primary fixing solution, particularly 2% paraformaldehyde; a secondary fixing/permeabilizing solution, particulary 70% ethanol; washing solution(s); and a DNA binding stain, all of which would be needed to practice the method for determining the proportion of cells transfected with the sequence of interest that are undergoing apoptosis. However, it would have *prima facie* obvious to one of ordinary skill in the art to make and use a kit comprising the reagents, because the cited references teach that these components would be need or could be used to practice the method for determining the proportion of cells transfected with the sequence of interest that are undergoing apoptosis. One of ordinary skill in the art would have been motivated to make and use a kit comprising the named components because kits are convenient and provide greater ease of practice.

Conclusion

17. No claims are allowed.

18. The prior art made of record and not relied upon is considered pertinent to Applicants' disclosure and may be used as the basis of new rejections of the claims under 35 USC §§ 102 and/or 103 during subsequent prosecution.

19. Any inquiry concerning this communication or earlier communications from the examiner should be directed to Stephen L. Rawlings, Ph.D. whose telephone number is (703) 305-3008. The examiner can normally be reached on Monday-Thursday, alternate Fridays, 8:00AM-5:30PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Anthony C. Caputa, Ph.D. can be reached on (703) 308-3995. The fax phone numbers for the organization where this application or proceeding is assigned are (703) 308-4242 for regular communications and (703) 308-4242 for After Final communications.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Stephen L. Rawlings, Ph.D.
Examiner
Art Unit 1642

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slr
February 11, 2002